IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Patent application of
Timothy J. Cunningham

Serial No.: 10/714,699

Filed: November 17, 2003

Group Art Unit: 1649

Examiner: Hayes, Robert Clinton

For: A SMALL SURVIVAL-PROMOTING/IMMUNOMODULATORY PEPTIDE FOR TREATMENT OF BRAIN DAMAGE, NEURODEGENERATIVE DISORDERS, AND INFLAMMATORY DISORDERS.

Declaration of Timothy J. Cunningham Under 37 C.F.R. 1.132

1. I, Timothy J. Cunningham, am a named inventor of the above-identified patent application. I am a Professor in the Department of Neurobiology and Anatomy of Drexel University in Philadelphia, PA.

2. The experimentation described in this declaration was conducted by me, or under my supervision. Drawings useful in the explanation of the data detailed in this declaration are attached as Exhibit A.

3. I have demonstrated that the CHEC-9 peptide is useful as a therapeutic agent in a rodent model of Multiple Sclerosis (MS). Dark Agouti (DA) rats were immunized with guinea pig myelin basic protein to establish experimental autoimmune encephalitis (EAE), a model of MS. Rats were treated with either CHEC-9 (n= 10) or vehicle (n = 10) for 10 days, starting 5 days after immunization. The treatment resulted in a highly significant inhibition of the disease (Figure 1) and accompanying spinal cord pathology (Figure 2).

4. EAE rats have markedly elevated systemic SPLA2 activity. Peak urinary activity correlates with the appearance of symptoms. CHEC-9 significantly reduces sPLA2 activity on days 10 and 12 post-immunization in the peptide-treated group. This time period corresponds to the onset of symptoms in the control (vehicle-treated) group. (Figure 3).

5. MS patients have markedly elevated systemic sPLA2 activity. As seen in the rodent EAE model, MS patients also have elevated sPLA2 enzyme levels, with the highest levels among symptomatic patients, i.e. during relapse or "active" MS (Figure 4). The level of enzyme activity in the urine of MS patients with active (N = 15) or stable (N = 29) disease is increased as compared to controls (N = 14. $p = 0.0019^{**}$, $p = 0.049$, respectively). All measurements were normalized to both total protein in each sample and to pre-immunization (for rats) or average control (for patients) values.

6. I have demonstrated that the CHEC-9 peptide is useful as a therapeutic agent in a rodent model of Amyotrophic Lateral Sclerosis (ALS). ALS patients have high levels of systemic sPLA2 activity (Figure 5). Likewise, sPLA2 activity is increased in G93ASOD1 mice, a mutant transgenic mouse model of ALS, compared with littermates that do not possess the transgene. This mutant is characterized by progressive hindlimb paresis/paralysis and motor neuron loss similar to ALS (Figure 6).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

10/31/07
(date)

Timothy J. Cunningham, Ph.D.

EXHIBIT A

Figure 1, comprising a graph depicting the effect of CHEC-9 treatment on EAE symptoms in DA rats immunized with guinea pig myelin basic protein/CFA. CHEC-9 treatment resulted in a significant inhibition of the disease symptoms, as indicated by the mean clinical score when compared with controls, and accompanying spinal cord pathology.

Figure 2 comprising Figure 2A and Figure 2B, depicts a pair of photomicrographs comparing the effects of CHEC-9 and vehicle control on spinal cord pathology in EAE rats. Photomicrographs show the region of the conus medularis (CM) in the spinal cord stained with a Nissl-myelin stain. Figure 2A is a photomicrograph of spinal cord pathology observed in an EAE rat treated with CHEC-9. Figure 2B is a photomicrograph of spinal cord pathology observed in a vehicle-treated EAE rat. Both rats were sacrificed 18 days after immunization with guinea pig myelin basic protein/CFA. As seen in Figure 2B, there is extensive spinal cord and cellular atrophy, cell infiltration, and large areas of myelin degeneration in the vehicle-treated rats with mean clinical scores of 2.0 and above (note scale bars are equal suggesting extensive collapse in this region of the spinal cord).

Figure 3 is a graph depicting the effect of CHEC-9 on urinary sPLA2 in EAE rats. Urinary sPLA2 levels peak just prior to the onset of EAE symptoms in this model of MS. CHEC-9 inhibition of sPLA2 activity, shown here as a percent of activity measured pre-immunization with guinea pig myelin basic protein/CFA, is also most apparent just prior to the onset of EAE symptoms, as would be expected if its action depended on a sufficient level of sPLA2 activity. A significant reduction in sPLA2 activity was observed on days 10 and 12 post-immunization in the peptide treated group which corresponded to the onset of symptoms in the control group.

Figure 4 is a graph depicting urinary sPLA2 activity in MS patients. MS patients have elevated sPLA2 levels, with the highest levels among symptomatic patients, i.e., during relapse or "active" MS (top bar). Level of enzyme activity in urine of MS patients with active (top bar; n=15) or stable (middle bar; n=29) disease is increased compared to controls (bottom bar; n=14, p=0.0019**, p=0.049 respectively).

Figure 5 is a graph depicting systemic sPLA2 activity in ALS patients (top bar) as compared to controls (bottom bar).

Figure 6 is a graph depicting sPLA2 activity in a model of ALS (G93A SOD1 transgenic mice). Increased sPLA2 activity urine of G93A/SOD1 mutant mice. Twenty-50µl samples of urine were collected at one pre-symtomatic stage (36 days (n=8), females only) and two symptomatic stages (80-85 and 129-134 days) and pooled according to the gender of the mice.